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Altitudinal variation of demographic life-history traits does not mimic latitudinal variation in natterjack toads (*Bufo calamita*)

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ABSTRACT

In anuran amphibians, age- and size-related life-history traits vary along latitudinal and altiudinal gradients. In the present study, we tested the hypothesis that altitudinal and latitudinal effects cause similar responses by assessing demographic life-history traits in nine *Bufo calamita* populations inhabiting elevations from sea level to 2270 m. Skeletochronologically determined age at maturity and longevity increased at elevations exceeding 2000 m, but female potential reproductive lifespan (PRLS) did not increase with altitude, as it did with latitude. Integrating the available evidence, it was found that lifetime fecundity of natterjacks decreased at the upper altitudinal range because PRLS was about the same as in lowland populations but females were smaller. In contrast, small size of northern females was compensated for by increased PRLS which minimised latitudinal variation of lifetime fecundity. Thus, this study provides evidence that altitudinal effects on life-history traits do not mimic latitudinal effects. Life-history trait variation along the altitudinal gradient seems to respond directly to the shortening of the annual activity period. As there is no evidence for increasing mortality in highland populations, reduced lifetime fecundity may be the ultimate reason for the natterjacks' inability to colonise elevations exceeding 2500 m.

1. Introduction

Life-history traits such as age at maturity, longevity and larval growth rates show a significant co-variation with altitude and latitude in many amphibian species (Morrison and Hero, 2003; Marquis and Miaud, 2008). Multiple proximate causes such as genetic adaptation and/or phenotypic plasticity may underlie this pattern (Roff, 2001; Sultan and Spencer, 2002). Divergent selection among local environments and restricted gene flow can cause populations to evolve in response to specific ecological conditions, leading to local adaptation. Phenotypic plasticity is an evolutionary factor that can promote or retard genetic change (Price et al., 2003; Marquis and Miaud, 2008). In anuran amphibians, conspecific populations along a wide altitudinal range vary in several life-history traits, but except for *Rana sylvatica* (Berven, 1982a,b) it remains mostly unclear whether this variation is based on genetic adaptation or phenotypic plasticity.

Typical demographic life-history traits affected by altitude and latitude are age at maturity and longevity. Delayed sexual maturation at high elevations is common in most species (e.g., *Pleurodema thaul*, Iturra-Cid et al., 2010), often associated with a subsequent increase in longevity (e.g., *Amolops mantzorum*: Liao and Lu, 2010a; *Bufo bufo*: Hemelaar, 1988; *Hyla annectans*: Liao and Lu, 2010b; *Nanorana parkeri*: Ma et al., 2009a; *R. sylvatica*: Berven, 1982a,b; *Rana temporaria*: Ryser, 1996; Miaud et al., 1999; *Rana swinhoana*: Lai et al., 2005; *Rana chensinensis*: Lu et al., 2006; Ma et al., 2009b). Corresponding effects on body size seem to differ among species (Morrison and Hero, 2003). A positive correlation between size and altitude was detected in *B. bufo* (Hemelaar, 1988), *R. chensinensis* (Lu et al., 2006; Ma et al., 2009a), *Rana sauteri* (Lai et al., 2003), *R. swinhoana* (Lai et al., 2005), *P. thaul* (Iturra-Cid et al., 2010), a negative correlation in *R. temporaria* (Ryser, 1996, but see Kozlowska, 1971 for a positive correlation and Elmberg, 1991 for no correlation), *Rana muscosa* (Matthews and Miaud, 2007), *Rana nigromaculata* (Liao et al., 2010), and *N. parkeri* (Ma et al., 2009b), whereas no correlation was detected in *A. mantzorum* (Liao and Lu, 2010a) and *H. annectans* (Liao and Lu, 2010b). Available evidence suggests that altitudinal effects on age and size are selected for independently and that anuran species differ considerably in magnitude of response. As life-history theory predicts that latitudinal and altitudinal effects cause similar demographic responses in amphibians (Morrison and Hero, 2003), proximate mechanisms modulating the latitudinal variation of the life-history traits age and size may determine altitudinal variability as well. To our knowledge, this prediction has not yet been tested in a single species covering the complete latitudinal and altitudinal ranges.

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Therefore, we aimed to test the hypothesis of common proximate mechanisms of age and size variation along geographical gradients in the natterjack toad, *Bufo* (=*Epidalea*) *calamita*. This anuran qualifies as a model organism because of its wide latitudinal (36–58°) and altitudinal range (sea level to 2540 m) and its well-understood latitudinal variability of age and size at lowland localities (Leskovar et al., 2006; Sinsch, 2008; Sinsch et al., 2010). In males, age at maturity and longevity show a slight and insignificant tendency to increase with latitude, whereas longevity and, subsequently, potential reproductive lifespan (PRLS) are significantly augmented in females (Leskovar et al., 2006). In contrast, the pattern of associated size variability is not compatible with a latitudinal cline but suggests optimisation of lifetime fecundity by a counter-gradient selection of female size and PRLS (Sinsch et al., 2010). High rates of gene flow over large distances (Marangoni, 2006; Oromi et al., unpublished data) suggest that age-adjusted size variation in response to the environmental constraints is more probably due to phenotypic plasticity of a "general purpose genotype" than to local genetic adaption, but putatively neutral genetic differentiation among wild populations often confounds phenotypic and genetic variation (e.g., Pujol et al., 2008; Richter-Boix et al., 2010; Lind et al., 2011). If the response of life-history traits to altitudinal environmental variation mimics that along the latitudinal gradient, we predict that (i) female PRLS will increase because the delayed age at maturity will be outweighed by a stronger increase in longevity, and (ii) size will decrease concurrently because of the shortening of the annual growth period. The interaction of these trends would optimise lifetime fecundity at high-elevation sites. We test these specific predictions by analysing trait variation in nine natterjack populations inhabiting the Sierra de Gredos and neighbouring lowland sites in Spain along an elevation range from 10 m to 2270 m.

2. Materials and methods

2.1. Populations and study sites

A total of 252 reproductive natterjack toads were studied at seven localities ranging from 10 m to 2270 m altitude in Spain. Habitat and local climate features, collection dates and numbers of reproductive adults sampled are summarised in Table 1. Five populations (Navaluenga = NL, La Dehesa del Barraco = DB, La Cedrera = CE, Cavadores = CA and Navasomera = NS) are situated in the Sierra de Gredos (Central Iberian System). The breeding sites of *B. calamita* in these localities are humid meadows and ponds at the lower sites, and glacial lagoons at the high-montane sites. Two populations (Palamós = PA, El Pinós = PI) are located outside the Sierra de Gredos (Table 1). The PA breeding sites are ephemeral ponds in a dune system, 1 km from the Mediterranean coast. El Pinós is situated in the Sierra del Reclot, 3.8 km from the Mediterranean coast, and toads reproduce in ephemeral water bodies or artificial ponds. To complement the analysis of altitudinal variation, we included data on two populations inhabiting the Ebro valley (Balaguer = BA, Mas de Melons = MM; Table 1) published in Leskovar et al. (2006) and Sinsch et al. (2007).

Climate variables used were minimum and maximum annual air temperature, average annual air temperature, and precipitation (mm/year). Temperature (15-year averages) and precipitation data (20-year averages) for the nine study areas were derived from the "Atlas Climático Digital de la Península Ibérica" (Ninyerola et al., 2005), except for precipitation data (30-year average) for BA and MM (meteorological station of Lleida, details in Leskovar et al., 2006). Substrate of study areas was classified either as "rock" (i.e., predominantly bare rock with crevices and little vegetation), "clay" (i.e., fine-grained minerals forming near impermeable soils), or "sand" (i.e., coarse-grained soil with more than 50% of material retained by a 0.075 mm sieve).

2.2. Sampling procedure and skeletochronological age estimation

Toads were collected at the local breeding ponds during the spring reproduction period of 2004–2010 (Table 1). The variation in sex ratio among populations is due to the fact that collecting surveys were not exhaustive and thus, samples represent a random selection of each local breeding assemblage. Toads were released in situ following sex determination, measuring of snout–vent length (SVL, to the nearest mm) and toe-clipping (3rd toe of the right hind limb). The toes were stored in 70% ethanol at room temperature.

Laboratory protocols for skeletochronology followed the procedure described by Tejedo et al. (1997). The samples were decalcified in 3% nitric acid for at least 20 min. Cross-sections (16 μm) were prepared using a freezing microtome (HM 50 N; Microm International GmbH, Walldorf, Germany) and stained with Ehrlich's hematoxyline (Sierra de Gredos populations) or cresylviolet (PA, PI). Cross-sections were examined for the presence of growth marks (strongly stained lines in the periosteal bone) using a light microscope (BX 50; Olympus, Tokyo, Japan) at magnifications of 400×. We selected diaphysis sections in which the size of the medullar cavity was at its minimum and that of the periosteal bone at its maximum.

We distinguished three types of growth marks: (i) lines of metamorphosis (produced by the physiological changes during metamorphosis), (ii) annual growth marks (lines of arrested growth (LAGs) = indicators of hibernation), and (iii) non-annual faint lines (indicators of occasional interruptions of hibernal inactivity periods). The number of annual growth marks (LAGs) was assessed by the first author and independently by one or two co-authors to estimate age as the number of completed hibernations. Non-annual growth marks were distinguished from annual ones using the method described by Sinsch et al. (2007) to calculate the percentage of individuals per population which showed histological equivalents of interrupted hibernation.

Minimum age at maturity was defined as the age of the youngest reproductive individual recorded at each locality, whereas the median age indicated the age class which included the greatest number of individuals. The median age was calculated as a proxy of population-specific lifespan. Longevity was defined as the maximum number of LAGs counted in an individual in each population. Potential reproductive lifespan (PRLS) was calculated as the difference between longevity and minimum age at maturity.

2.3. Statistical analysis

All variables were first tested for normality. The influence of altitude on the percentage of multiple lines per population and on local age variation was estimated by fitting data in 29 regression models. We chose the reciprocal-Y squared-X model for multiple line prediction and the squared-X model for age prediction because they provided the best fit, i.e., maximum R^2. As age and SVL distributions were significantly skewed, data were normalised by log(10)-transformation. A factorial analysis of log-normalised age and size data and the corresponding environmental factors was conducted to obtain a small number of statistically independent factors which account for most of the variation of the original variables. An orthogonal varimax rotation was performed to maximise the sum of the variances of the squared loadings, i.e., a rotation procedure was used to approximate the factor loadings of individual variables either close to 0 or to ±1. Potential sexual dimorphism